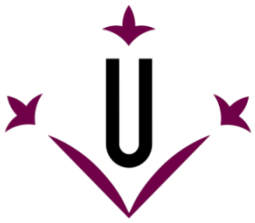

Enhancing phenolic content in carrots by pulsed electric fields during post-treatment time: effects on cell viability and quality attributes

Gloria López-Gámez [a], Pedro Elez-Martínez [a], Olga Martín-Belloso [a], Robert Soliva-Fortuny [a*]

[a] Department of Food Technology, Agrotecnio Center, University of Lleida, Av. Alcalde Rovira Roure, 191, 25198 Lleida, Spain

* Corresponding author: Fax: +34 973 702596; e-mail: rsoliva@tecal.udl.cat

Abstract

The impact of pulsed electric fields (PEF) and post-treatment time on the phenolic content and quality attributes of carrots was studied. Additionally, their influence on cellular permeability and viability was analysed. Carrots were subjected to different electric field strengths (0.8, 2 and 3.5 kV·cm−1) and number of pulses (5, 12 and 30). The largest increases in phenolic content were produced 24 h after applying 30 pulses of 0.8 kV·cm-1 (40.1%) and 5 pulses of 3.5 kV·cm-1 (39.5%). At such conditions, the colour was not affected but softening occurred after applying the highest electric field strength. Moreover, the increase in the specific energy input correlated with the decrease in cell viability. Carrot weight loss over time, media conductivity increase and cell viability decrease are related to the destabilization of cell membranes, which would entail a physiological response to stress, leading to a higher content in phenolic compounds.

Keywords: Pulsed electric field; polyphenols; quality attributes; cell viability; carrot

1. Introduction

Carrot is an economically important crop whose consumption is becoming increasingly popular due to its nutritional value. Carrots are known as a good source of bioactive compounds such as carotenoids, phenolic compounds, vitamin C and fiber, among others (Arscott & Tanumihardjo, 2010). Most of the available information regarding their phytochemical content is related to carotenoids and their antioxidant properties, whereas their phenolic content has been less studied. Hydroxycinnamic acids, such as chlorogenic acid (CHA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) are the main polyphenols accumulated in carrots (Becerra-Moreno et al., 2015), resulting from the induction of the phenylpropanoid pathway, as a plant defence response to stress (Zhao, Davis, & Verpoorte, 2005). Plant growth is affected by environmental factors such as extreme temperatures, drought, nutrients imbalance, wounding (abiotic stresses) or infections by pathogenic organisms (biotic stresses). Nevertheless, plants have developed adaptation mechanisms, e.g. the synthesis of secondary metabolites such as polyphenols. Heredia & Cisneros-Zevallos (2009) reported that carrots showed a higher antioxidant activity compared to other fruits and vegetables in response to wounding. In that sense, it was concluded that more phenolic compounds were accumulated in treatments that involved the most intensive damage. Some studies have reported that polyphenol biosynthesis and accumulation in plant tissues may be induced through the application of postharvest abiotic stresses such as wounding (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015) or UV-light radiation (Aguiló-Aguayo, Gangopadhyay, Lyng, Brunton, & Rai, 2017; Alegria et al., 2012), among others.

Recently, the application of non-thermal processing technologies such as pulsed electric fields (PEF) has been proposed as an innovative stress promoter to accumulate bioactive compounds (Jacobo-Velázquez et al., 2017). PEF consists on delivering short high-voltage bursts of electrical energy to a food placed between two electrodes. PEF application induces a transmembrane potential difference across the cell membrane. A phenomenon known as electropermeabilization occurs when this potential reaches a critical value, which results in

63 increasing the cell permeability (Knorr & Angersbach, 1998) and the breakage of the membrane
64 in a reversible or irreversible way. As a response, several metabolic and structural changes are
65 triggered in plant cells, which may have an impact on quality attributes and bioactive
66 compounds content. PEF treatments have exhibited the potential of maintaining the physico-
67 chemical quality of liquid food products inactivating microorganisms and enzymes without
68 significantly impacting their properties (Martín-Belloso & Elez-Martínez, 2005), enhancing
69 intracellular metabolite extraction or improving the drying efficiency (Soliva-Fortuny, Balasa,
70 Knorr, & Martín-Belloso, 2009). However, the application of PEF as an abiotic stressor for the
71 biosynthesis of secondary metabolites in fresh produce is a research area still under
72 development. PEF parameters must be optimized to avoid undesirable effects on sensorial or
73 nutritional characteristics. In this sense, it is important to understand how PEF affects the
74 viability of plant cells and to evaluate its uniformity across tissues. The effects of PEF on
75 quality attributes have been independently studied of those caused on bioactive compounds. It
76 has been reported that PEF may induce softening of carrot tissues and reduce their cutting
77 resistance (Lebovka, Praporscic, & Vorobiev, 2004; Leong & Oey, 2014; Wiktor et al., 2018) as
78 well as modify their colour (Wiktor et al., 2015). There are only few reports assessing non-
79 thermal processing effect on bioactive compounds in carrots [e.g. ultrasounds (Cuéllar-
80 Villarreal et al., 2016; Nowacka & Wedzik, 2016) or UV radiation (Formica-Oliveira, Martínez-
81 Hernández, Díaz-López, Artés, & Artés-Hernández, 2017)]. An enhancement in the
82 extractability of carotenoids from carrot slices after PEF was achieved after applying 1.85
83 $kV \cdot cm^{-1}$ and 10, 50 and 100 pulses (Wiktor et al., 2015). There are also studies in other whole
84 products: potato (Galindo et al., 2009), apple (Soliva-Fortuny, Vendrell-Pacheco, Martín-
85 Belloso, & Elez-Martínez, 2017), and tomato (Vallverdú-Queralt et al., 2013). However, the
86 effect of PEF on both bioactive content and quality attributes were only studied in blueberry
87 (Jin, Yu, & Gurtler, 2017) and tomato (González-Casado, Martín-Belloso, Elez-Martínez, &
88 Soliva-Fortuny, 2018). As far as we know, there is no available information about the effect of
89 PEF and post-treatment time on the phenolic content and on the quality attributes of whole
90 carrots. The identification of the optimal conditions that enhance phenolic content without

altering the quality of carrots provides an opportunity to obtain derived products with health-promoting properties and meet the consumer demand for natural and functional products.

The central idea behind this study was the application of PEF treatments as a strategy to enhance the phenolic content of carrots. In addition, their effects on quality attributes and PEF impact on cell viability and permeability were determined.

2. Material and methods

2.1. Chemicals and reagents

HPLC grade methanol and analytical reagent grade sodium carbonate were acquired from Fisher Scientific Scharlau Chemie (Loughborough, UK), 2, 3, 5-triphenyltetrazolium chloride was purchased from Sigma-Aldrich (St. Louis, MO, USA), Folin-Ciocalteu reagent was acquired from Scharlau S.L. (Barcelona, Spain) and sodium chloride was purchased from POCH S.A. (Sowińskiego, Poland).

2.2. Carrots sample

Carrots (*Daucus carota* cv. Nantes) were obtained in a local supermarket in Lleida (Spain) and were stored at 4 ºC for less than a week until treatment. Whole carrots with caliber 25/35 mm and length of 17 ± 2 cm were washed with tap water and dried carefully with a paper cloth before application of PEF treatments.

2.3. Pulsed electric fields (PEF) treatments

PEF treatments (**Table 1**) were conducted in a batch PEF system (Physics International, San Leandro, CA, USA). The equipment delivers pulses with exponentially decaying waveform pulses of 4 μs from a capacitor of 0.1 μF at a frequency of 0.1 Hz. The time interval between pulses was $\Delta t = 2$ s. It is equipped with a TG-70 gas control unit and a PT55 pulse generator (Pacific Atlantic Electronics Inc., El Cerrito, CA, USA). The pulse generator had a charge voltage of +5 to +7 kV DC, a trigger pulse of +250 V and an output voltage of +50 kV. The treatment chamber consisted of a parallelepiped methacrylate container with two parallel stainless steel electrodes (20 × 5 cm) separated by a gap of 5 cm. Carrots were placed in parallel to the

electrodes and immersed in an aqueous solution (conductivity of 10 μS·cm^{-1}). Different electric field strengths (0.8, 2 and 3.5 kV·cm^{-1}) and number of pulses (5, 12 and 30 pulses) were applied in accordance to Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso (2009). The specific energy input (Ws), expressed in kJ/kg, delivered with each treatment was calculated according to Eq. (1):

$$Ws = \frac{V^2 \cdot C \cdot n}{2 \cdot m} \quad (1)$$

where V [V], C [F], n, and m [kg] are the voltage, capacitance of the energy storage capacitor, number of pulses and mass of the sample in the treatment chamber, respectively. The temperature of the treatment aqueous solution after PEF application was below 20.0 °C. The mass of the sample was equal to about 0.1 kg and the ratio carrots:aqueous solution was 1:3 (w:v).

2.4. Cell permeability

Electrical conductivity. The electrical conductivity of the aqueous solution contained in the treatment chamber was measured before and after PEF treatments using a conductometer Testo 240 (Lenzkirch, Germany). The solution was replaced after each treatment and measures were performed twice.

Weight loss. Carrots weight loss was evaluated after PEF treatments. Gravimetric methods were used [AND electronic Balance FX-2000 (AND Company Limited, Tokyo, Japan)] and measures were performed twice. Weight loss was calculated in reference to untreated carrots with the Eq. (2).

$$Weight\ loss\ (\%) = \frac{W_0 - W}{W_0} x\ 100 \quad (2)$$

Where W_0 is referred to weight of untreated carrots and W is referred to weight of PEF-treated carrots (just after, 24 h and 48 h after treatment). Lag time between treatment and weight measurement just after treatment averaged 20 minutes.

22.5. Cell viability

Tetrazolium salt staining was used to evaluate the effect of PEF on cell/tissue viability within carrot slices and to determine the proportion of viable cells, as previously described by Faridnia, Burritt, Bremer, & Oey (2015). The principle of tetrazolium salt staining is based on the formation of an insoluble red formazan from the reduction of the salt by oxidoreductase enzymes. The amount of formazan is directly proportional to the number of living cells as they contain oxidoreductases (Berridge, Herst, & Tan, 2005). A 0.5% (w/v) tetrazolium salt solution was prepared with Milli-Q water on the same day as the PEF treatment. Immediately after PEF treatment, each carrot was sliced (1.5 mm thickness and 32 ± 3 mm diameter). Three slices of each carrot were placed in a petri dish and fully immersed in the tetrazolium solution. Petri dishes were then covered in tin foil to protect the samples from light and left for 24 h at 18–20 °C. After 24 h slices were rinsed with water, blotted dry with a paper towel and photographed. Image analysis using colour threshold method [ImageJ software, (Abràmoff, Hospitals, Magalhães, & Abràmoff, 2007)] was applied to measure the red and unstained areas on each slice, indicative of living and dead cells, respectively. Cell viability (%) of each slice was calculated using the following Eq. (3):

$$Cell\ viability\ (\%) = \frac{Stained\ area}{Total\ area} x100 \quad (3)$$

Measures were conducted in three slices for each treatment replicate.

2.6. Post-treatment determinations

Carrots were treated with different PEF conditions in order to evaluate their effect on the total phenolic content and over their quality attributes immediately, 24 h or 48 h at 4 ºC after PEF treatments.

2.6.1. Extraction and analysis of phenolic compounds

Phenolic compounds were extracted following the methodology proposed by Ribas-Agustí, Cáceres, Gratacós-Cubarsí, Sárraga, & Castellari (2012) with slight modifications. Carrot tissue (5 g) was homogenized with an 80% (v/v) methanol solution (25 ml) using an Ultra-Turrax T25 (IKA® WERKE, Germany). Homogenates were centrifuged at 21612 *g* at 4-6 °C for 20 min. Supernatants were collected and 25 ml of methanol solution was added to the precipitate. In order to ensure a complete extraction, precipitates were vortexed for resuspension and immersed in an ultrasonic bath for 5 minutes. Samples were centrifuged under the same conditions explained above. Both supernatants were collected and stored at -40 ºC (for up to ten days until analysis).

The total phenolic content was determined according to the Folin–Ciocalteu procedure adapted to 96-wells microplates. Methanolic extracts (30 µl) were placed into a microplate. Then, 150 µl of 10% (v/v) Folin-Ciocalteu's reagent and 120 µl of Na_2CO_3 7.5% (w/v) were added. After an incubation period of 90 min at room temperature in darkness, the absorbance was measured at 765 nm using a microplate reader (Thermo Scientific Multiskan GO, Vantaa, Finland). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight relative to those of untreated carrots. Phenolic content was extracted twice per replica and spectrophotometrically determined four times.

2.6.2. Quality attributes

Hardness. Hardness of cortical tissue and vascular cylinder of carrots were determined in three carrot disks (15 mm height and 32 ± 3 mm diameter) of each replica. These disks were cut out of top end (1/3 of total length) of the carrot. Hardness was determined with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England), equipped with a 4-mm-diameter cylinder steel probe, which penetrated 10 mm the carrot tissue at a constant rate of 5 $mm \cdot s^{-1}$. The motion of the blade was perpendicular to the surface of carrot disks. Hardness (N·s) was determined as area under the curve between the graph of y (force) and x (time).

Colour. The CIELab parameters (lightness, L*; green-red chromaticity, a*; and blue-yellow chromaticity, b*) were utilized to characterise the external colour of carrots from each treatment using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The apparatus was set up for a D65 illuminant and 10° observer angle. A white standard plate (Y=94.00, x=0.3158, y=0.3322) was used for calibration. The colour was assessed by measuring the lightness (L*), the a*/b* ratio and total colour difference (ΔE), calculated by Eq. (4).

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{0.5} \quad (4)$$

Where L, a and b refer to data collected after treatments and L_0, a_0 and b_0 refer to untreated carrots. Three readings were made in each replica by changing the position of the carrot in each measure.

2.7. Statistical analysis

Statistical analyses were carried out using the JMP Pro v.13 software (SAS Institute, Cary, NC, USA) and IBM SPSS Statistics 24 (SPSS Inc., Chicago, IL). There were three different replicates for each assayed treatment condition and each analysis was conducted at least twice. Results were reported as the mean ± standard deviation. Normality and homocedasticity criteria were evaluated by Shapiro-Wilk and Levene tests, respectively. Results were subjected to an analysis of variance (ANOVA) followed by Tukey post hoc test in order to establish statistical differences among mean values. Repeated measures ANOVA was applied to establish differences among tissues hardness. The relationship between variables was determined using the Pearson (r) coefficient. Rho of Spearman (r_s) was used to establish a correlation between the specific energy input and media conductivity because of the outliers presence. The statistical significance level was set up at $p < 0.05$.

3. Results and discussion

3.1. Effects of PEF on cell permeability

In the present study, electroporation efficiency was evaluated based on changes in media conductivity immediately after treatments (**Table 1**) and in carrots' weight throughout 48 h after applying PEF treatments in a range of energies (0 - 3.93 kJ/kg).

Conductivity of the media in which carrots were immersed during treatments significantly ($p < 0.05$) increased after applying electric field intensities of 2.0 $kV \cdot cm^{-1}$ or 3.5 $kV \cdot cm^{-1}$. The obtained results suggest that the main factor affecting conductivity was the electric field strength, followed by the number of pulses and their interaction ($p < 0.001$). The specific energy input has also influence in the conductivity increase, as a significant ($p < 0.001$) correlation ($r_s = 0.774$) between those factors was found (**Fig. 1**). However, the increase in pulse number only caused an increment in media conductivity when 3.5 $kV \cdot cm^{-1}$ treatments were applied. Variations in pulse number and electric field strengths are highly related to changes in the specific energy input (Eq. (1)). The influence of pulse number was likely detected in 3.5 $kV \cdot cm^{-1}$ treatments because the range of energy studied at this electric field strength is broader than that of 0.8 $kV \cdot cm^{-1}$ and 2 $kV \cdot cm^{-1}$ treatments.

It is well known that the application of PEF to cell tissues results in electropermeabilization, which leads to release intracellular content, mainly ions and charged particles to the surrounding medium, increasing its conductivity and promoting weight loss. However, few studies have reported results using this approach in whole products. In order to electropermeabilize carrots, Bazhal, Lebovka, & Vorobiev (2003) determined an optimal value of 2.5 ± 0.2 $kV \cdot cm^{-1}$. Similar results were reported by Wiktor et al. (2015), who established that 1.85 $kV \cdot cm^{-1}$ was not enough for that purpose. Reversibility generally occurs when only a small portion of the membrane has pores (Knorr & Angersbach, 1998) or the total membrane area is bigger than the electropermeabilized zone, which enables cells to repair themselves. Obtained results demonstrate that higher field strengths caused higher cell disruption and suggest that 0.8 $kV \cdot cm^{-1}$ treatments were probably insufficient to form pores or cause irreversibility. On the other hand, more intense treatments caused an increase in conductivity because of the release of intracellular content, probably associated to the formation of irreversible pores. This increase in

conductivity may be consequence of a better water transference. In this sense, Aguiló-Aguayo et al. (2014) confirmed that cytoplasmic content of PEF-treated carrots was mixed with the extracellular liquid after treatments. Furthermore, the formation of pores caused by PEF enhances the diffusion of low molecular weight compounds such as fructose, glucose and sucrose (Janositz, Noack, & Knorr, 2011) and losses of ions, such as Ca^{2+} or K^{+} (Faridnia et al., 2015), into the medium by passive processes.

Some PEF treatments and post-treatment time caused a significant ($p < 0.05$) decrease in carrots weight. The electric field strength applied was the main PEF processing parameter affecting weight loss ($p < 0.001$), followed by the interaction with the number of pulses ($p < 0.001$). Furthermore, a strong and direct correlation was found between the specific energy input and weight loss of carrots immediately after treatments ($r = 0.820$; $p < 0.001$). **Fig. 2** shows that weight decreased throughout 48 h, specifically after the most intense treatments. Weight loss was observed immediately after applying energies higher than 0.5 $kJ \cdot kg^{-1}$ ($E \geq 2$ $kV \cdot cm^{-1}$). In addition, differences 24 h after applying 2 $kV \cdot cm^{-1}$ and 3.5 $kV \cdot cm^{-1}$ treatments were also noticeable. Carrots treated by energies higher than 0.61 $kJ \cdot kg^{-1}$ ($E = 3.5$ $kV \cdot cm^{-1}$) showed remarkable decreases in weight, reaching a maximum loss of 9.4 ± 1.3 % at 48 h. To the best of our knowledge, there are scarce studies evaluating weight loss of vegetable products after PEF treatments. The results suggest that the weight loss and the increase in media conductivity were highly correlated ($r = 0.931$; $p < 0.001$), meaning that changes in weight were mainly caused by the release of intracellular fluids due to electropermeabilization. Weight loss throughout time may be related to the formation of irreversible pores. Some authors reported that minimal processing (e.g. fresh-cut processing) increases the stress and respiration rate (Sandhya, 2010) causing structural changes as redistribution of water in tissues and cellular decompartmentalization, which could entail weight loss increase.

3.2. Influence of PEF treatment on cell viability

The impact of PEF on cell viability of carrots was evaluated after staining carrot slices with tetrazolium salt. The staining pattern (**Fig. 4**) was not homogeneous throughout PEF-treated

carrot tissues and among treatments. The effect of PEF depends on size, shape, orientation or electric properties of cells as observed by Faridnia, Burritt, Bremer, & Oey (2015) in PEF-treated potatoes. Furthermore, different physiological types of cells are generated after applying PEF: dead cells (membrane integrity and metabolic activity lost), intact cells (intact membrane and metabolic activity) and sublethally injured cells (SICs) (partial loss of membrane integrity and intact metabolic activity). Then, it must be taken into account that SICs and intact cells cannot be differentiated with this type of tinction.

A correlation between the specific energy input and cell viability was found (r = - 0.706; p < 0.01). Nevertheless, decreases in cell viability fitted better to increments in the electric field strength (**Table 3**). Results obtained indicate that electric field strength was the main parameter affecting cell viability (p < 0.001), followed by its combination with the number of pulses (p < 0.01). Carrots treated with an electric field strength of 0.8 kV·cm^{-1} showed mainly live cells with a similar pattern as those untreated, indicating that pores were not formed or their formation was reversible under these conditions. Formed pores must be small in comparison to total area of the cell membrane, therefore carrot cells were able to repair themselves and maintain their integrity. At this electric field strength, cell viability was maintained (91.1 - 84.1%) regardless the number of pulses applied. These results are in accordance with the insignificant weight loss (**Fig. 2**) and the maintenance in the conductivity of the medium (**Fig. 1**) in the same treatment conditions. On the other hand, after 2 kV·cm^{-1} and 3.5 kV·cm^{-1} treatments, an increase in media conductivity was observed and an immediate weight loss was reported after the most intense. In this sense, after applying 2 kV·cm^{-1}, cell viability was significantly lower (81.0 – 73.5%) than that of untreated ones. Dead cells were mainly observed in the cortical parenchyma when 5 and 12 pulses were applied (0.22 and 0.50 kJ·kg^{-1}, respectively), which may be explained due to different cell properties between tissues such as cell size. Vascular cylinder is composed by smaller cells; therefore, a higher electric field strength should be applied to achieve their electroporation. Interestingly, after applying 30 pulses (1.19 kJ·kg^{-1}), the pattern was more similar to those obtained in 3.5 kV·cm^{-1} treatments.

289 Despite the qualitative dissimilarities, quantitative data did not show significant differences
290 among 2 kV·cm^{-1} treatments with different number of pulses. After applying 3.5 kV·cm^{-1},
291 viability significantly ($p < 0.05$) decreased (87.5 - 79.4%). As shown in **Fig. 4**, the epidermis
292 was the most affected tissue, which at least had metabolic activity in these conditions.
293 According to Jacobo-Velázquez et al. (2017), damaged cells (in this case, epidermis cells)
294 would release ATP as a signaling molecule for undamaged cells (internal tissues), that would
295 trigger the activation of stress response leading to higher antioxidant activities to avoid damage.

296 **3.3. Effects of PEF and time post-treatment on quality attributes**

297 **3.3.1. Hardness**

298 Carrots have a complex tissue with different cell sizes and orientations, which may cause a
299 heterogeneous effect of PEF across the whole product. Hence, the influence of PEF treatments
300 on the hardness of carrots was investigated through a penetration test in cortical tissues and
301 vascular cylinder immediately, 24 h and 48 h after treatments.

302 Hardness was significantly ($p < 0.05$) different between both cortical tissues and vascular
303 cylinder depending on the treatment applied (**Fig. 3**). Statistical analysis indicated that the main
304 parameter affecting hardness was the electric field strength ($p < 0.01$), followed by the
305 interaction with the number of pulses ($p < 0.05$).

306 Differences among tissues treated under the same conditions were observed immediately after
307 treatments. Vascular cylinders were softer (26.50 % - 8.75 %) ($p < 0.05$) than the cortical
308 tissues. However, neither the hardness of the cortical tissue nor that of the vascular cylinder
309 were significantly ($p > 0.05$) affected in comparison to the same tissues in untreated carrots,
310 regardless the number of pulses applied. Differences among tissues observed just after
311 treatments were not maintained after 24 h. At such time, the hardness of the cortical tissue of
312 carrots treated by 30 pulses of 3.5 kV·cm^{-1} (3.93 kJ·kg^{-1}) was lower (8.3 ± 1.0 N·s) compared to
313 untreated carrots (11.4 ± 1.0 N·s), but the hardness of the vascular cylinder was similar among
314 treatments. After 48 h of applying 3.5 kV·cm^{-1} treatments, hardness decreased in both cortical

315 (8.60 - 9.12 N·s) and vascular tissues (7.40 - 8.39 N·s) compared to that of untreated ones
316 (cortical tissue: 11.5 ± 0.1 N·s; vascular cylinder: 8.6 ± 0.9 N·s). On the other hand, cortical
317 hardness of carrots treated by 2 $kV \cdot cm^{-1}$ increased after 48 h, in contrast to that obtained 24 h
318 after treatments, whereas vascular tissue remained similar to those untreated.

319 Regarding the differences among vascular cylinder and cortical tissues within the same
320 treatments, the effect of PEF conditions depends on numerous factors including cell properties
321 (size, conductivity, shape and orientation) and PEF treatments parameters (electric field
322 strength, pulse amplitude, shape, duration and number) (Vorobiev & Lebovka, 2009). Obtained
323 results suggest that changes observed throughout time were mainly due to permeabilization of
324 membranes in the most intense treatments. A decompartmentalization process, a water
325 redistribution across the tissues and a loss of turgor occurs immediately after PEF and
326 throughout post-treatment time (Aguiló-Aguayo et al., 2014). These results are in accordance
327 with the correlations found among hardness decrease and weight loss, decrease in lightness and
328 increase in media conductivity (Supplementary material). On the other hand, the hardness
329 increase in carrots treated by 2 $kV \cdot cm^{-1}$ after 48 h may be a result of lignification, a plant
330 defence mechanism to prevent water loss (Becerra-Moreno et al., 2015).

331 **3.3.2. Colour**

332 Colour is one of the most important quality parameters to be preserved in fruits and vegetables.
333 Certain changes in colour provides information related to tissue destructuration and consumers'
334 perception. Changes in colour were evaluated throughout time measuring of L*, a*/b* ratio and
335 ΔE.

336 The application of PEF and time after treatment contributed to significant ($p < 0.05$) changes in
337 colour. The main parameter responsible for this effect was the electric field strength ($p < 0.001$),
338 followed by its interaction with the number of pulses ($p < 0.05$). The application of higher
339 electric field strengths was associated with a significant ($p < 0.05$) decrease in lightness in
340 comparison to untreated carrots. As **Table 2** shows, 24 h after PEF, only carrots subjected to the

most intense treatments (12 and 30 pulses of 3.5 $kV \cdot cm^{-1}$) showed significantly decreased L* values (47 ± 4 and 47.0 ± 2.1, respectively) compared to those untreated (53.9 ± 2.4). Moreover, 48 h later, differences were noticeable in all PEF-treated carrots except for the 0.8 $kV \cdot cm^{-1}$ conditions, which did not cause significant changes in lightness throughout time. These results are in agreement with previous data suggesting that 0.8 $kV \cdot cm^{-1}$ treatments are insufficient to cause irreversible electropermeabilization. Changes in L* value are a consequence of decompartmentalization and cell membrane disruption, which favour the contact between oxidative enzymes, such as peroxidase (POD) and polyphenol oxidase (PPO) and their phenolic substrates, previously located in the vacuoles (Wiktor et al., 2015). A correlation ($p < 0.001$) was found between L* values corresponding to 24 h after PEF and the specific energy input applied ($r_s = -0.697$). These results suggest that initial decrease in lightness could be a direct consequence of electropermeabilization and cell disruption. However, the latest changes may be due to metabolic alterations induced by structural cell damage. Regarding the a*/b* ratio, it remained stable among treatments and over 48 h, indicating no differences in redness and yellowness, parameters that have been related to carotenoid content in tomato fruit (Arias, Lee, Logendra, & Janes, 2000).

The total colour difference (ΔE) was significantly ($p < 0.05$) affected by PEF treatments and post-treatment time. Immediately, and 24 h after treatment, differences were only significant after applying 12 pulses of 3.5 $kV \cdot cm^{-1}$ and 30 pulses of 2 $kV \cdot cm^{-1}$, respectively. However, after 48 h, a noticeable increase in comparison to untreated carrots was observed in carrots treated by 2 $kV \cdot cm^{-1}$ and 3.5 $kV \cdot cm^{-1}$. These variations are mainly related to L* value decrease explained above. It is remarkable that ΔE values were higher than 2 regardless the treatment applied and time after PEF. Values greater than 2 indicates that such colour change could be visible by a consumer with the naked eye (Tiwari, Patras, Brunton, Cullen, & O'Donnell, 2010). Therefore, subjecting carrots to PEF would modify the product appearance. Wiktor et al., (2015) determined similar effects on ΔE values of PEF-treated fresh-cut carrots, although a time effect after treatments on this parameter was not found. This fact might be related to some variations

between both studies. Measurements were made 60 min after PEF instead of 48 h in this study, mechanical damage caused by cylinder cutting instead of using the whole vegetable or the orientation of the carrot with respect to the electrodes (data not provided), which may explain the differences regarding the PEF effect on tissues (Faridnia et al., 2015).

3.4. Effects of PEF and time post-treatment on total phenolic compounds content

Results indicate that the application of PEF and post-treatment time significantly ($p < 0.001$) affected phenolic compounds content in carrots (**Fig. 5**). Statistical analysis suggests that post-treatment time and the electric field strength were the most influential factors followed by the interaction between electric field strength and the number of pulses.

No significant ($p > 0.05$) changes in phenolic compounds content were reported compared to untreated carrots immediately after PEF application. After 24 h of PEF, some treatments caused a significant ($p < 0.05$) increase in the phenolic content compared to that found in untreated carrots stored under the same conditions. Higher increases in comparison to untreated carrots were given after 5 pulses of 3.5 $kV \cdot cm^{-1}$ (0.61 $kJ \cdot kg^{-1}$) (39.5 ± 0.1 %) and 30 pulses of 0.8 $kV \cdot cm^{-1}$ (0.87 $kJ \cdot kg^{-1}$) (40.1 ± 0.2 %). The remaining treatments caused a minor increase, with the exception of 12 pulses of 2 $kV \cdot cm^{-1}$ (0.50 $kJ \cdot kg^{-1}$) and 30 pulses of 3.5 $kV \cdot cm^{-1}$ (3.93 $kJ \cdot kg^{-1}$), which remained unaltered. On the other hand, 5 and 12 pulses of 0.8 $kV \cdot cm^{-1}$ (0.14 and 0.38 $kJ \cdot kg^{-1}$, respectively) showed a decrease. After 48 h, a significant ($p < 0.05$) decrease in phenolic content occurred in carrots subjected to a field strength equal or higher than 2 $kV \cdot cm^{-1}$, whereas after the application of 0.8 $kV \cdot cm^{-1}$ phenolic content remained unaltered.

The maintenance of the phenolic content immediately after PEF suggests that these compounds were not released through the formed pores, although media conductivity (**Fig. 1**) and weight loss (**Fig. 2**) increased. Probably, PEF conditions applied were not enough to electroporate vacuoles, where phenolic compounds are mainly located. Galindo et al. (2009) reported that PEF-treated potato slices had a metabolic profile similar to that of untreated ones immediately after treatment. These results are consistent with the mechanism of action of plant stress

394 defence. As an immediate response, during the first minutes, stress signaling molecules are
395 produced [i.e. reactive oxygen species (ROS), ethylene, jasmonic acid, etc.] that would activate
396 the expression of genes involved in the primary and secondary metabolism of the plant. Because
397 of this activation, a long-term physiological process (hours or days) (Zhao et al., 2005) takes
398 place to adapt to the new environment or to recover from damage. For instance, increasing the
399 activity of certain enzymes, the biosynthesis of secondary metabolites (Jacobo-Velázquez et al.,
400 2015), causing changes in respiration rate or in carbon sources. Nonetheless, in this study, a
401 delayed defensive response must be considered. The increase in media conductivity after some
402 treatments may involve an insufficient level of Ca^{2+} and K^{+} in the cytoplasm, which are
403 important molecules to trigger the signaling network during stress response. These ions can be
404 released during PEF treatments through formed pores as reported by Faridnia, Burritt, Bremer,
405 & Oey (2015).

406 After 24 h of PEF treatments, variations in the phenolic content were observed among
407 treatments. These differences are difficult to explain due to the complexity of metabolic
408 networks in natural systems; several factors may be involved in these changes. Vallverdú-
409 Queralt et al. (2013) based on a PLS-DA analysis in PEF-treated tomatoes, reported that
410 changes in individual phenols were highly correlated to specific combinations between number
411 of pulses and electric field strengths, causing differences in the total content. On the other hand,
412 the type of formed pores and the way cells detect the damage intensity might cause these
413 changes. By increasing field strength, pulse duration and number of pulses, the number and size
414 of pores in the cell membrane increase (Bazhal et al., 2003; Janositz et al., 2011; Knorr &
415 Angersbach, 1998). After 0.8 $kV \cdot cm^{-1}$, transient small pores are probably more common than
416 irreversible ones. Hence, the observed decrease in phenolic content may be a plant defence way
417 to fastly obtain energy through degradation of starch or antioxidants compounds and contribute
418 to the hexose pool to recover from this weaker damage. More intense treatments (2 $kV \cdot cm^{-1}$ and
419 3.5 $kV \cdot cm^{-1}$) cause a strong structural damage (i.e. less intercellular adhesion, permanent pores
420 in membranes, intracellular content release). Furthermore, an increase in mass transfer may

cause osmotic dehydration, an additional stress that require a different metabolic strategy, which probably involves *de novo* biosynthesis of phenolic compounds to avoid oxidative damage. As a result, an increase in phenolic content was observed in these conditions.

A decrease of phenolic content in carrots was observed after 48 h of some PEF treatments. Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez (2017) reported similar results in apples submitted to PEF treatments and stored for 48 h at different temperatures. Several reasons would explain our results. Firstly, due to structural decompartmentalization, oxidative enzymes such as PPO or POD contact easily with their phenolic substrates. This idea is supported by obtained colour data (**Table 2**). L* value was decreased in the same treatments characterized by the lowest phenolic content at 48 h. In addition, a higher weight loss indicates a major intracellular liquid release and cellular destructuration that favoured this contact. Secondly, the decrease in phenolic compounds after 2 $kV \cdot cm^{-1}$ treatments may be related to lignification process given the intracellular liquid release and hardness maintenance in cortical tissues observed at this time. Becerra-Moreno et al. (2015) reported that the combination of water loss and wounding stress might entail the accumulation of phenolic compounds in plants depending on their biosynthesis and utilization rate for lignin biosynthesis. Finally, electrical pulses could affect the three-dimensional structure of proteins and enzymes, constituted by weak non-covalent forces, hydrogen bonds and hydrophobic interactions (Ohshima, Tamura, & Sato, 2007). In the most intense treatments, 3.5 $kV \cdot cm^{-1}$, the highest intracellular liquid release, weight loss and decrease in hardness suggest that phenolic compounds may be released through formed pores and damaged protein channels responsible of the active transport system. These structural changes may be useful to enhance the release of phenolic compounds of cells and improve their bioaccessibility.

4. Conclusions

Results reported in this study suggest that the electric field strength was the most influential variable in media conductivity, quality attributes, cell viability and phenolic content variations. Additionally, a high correlation was found among media conductivity and the specific energy

input. Moreover, PEF and post-treatment time affected the amount of phenolic compounds in carrots as well as their quality attributes. Carrots treated by 5 pulses of 3.5 $kV \cdot cm^{-1}$ or 30 pulses of 0.8 $kV \cdot cm^{-1}$ and stored during 24 h at 4ºC led to the highest increases (39.5% and 40%) in phenolic content compared to those in the untreated carrots. At such conditions, surface colour was maintained, but weight loss and softening occurred because of electropermeabilization and cell disruption promoted by the most intense PEF condition. Irreversible damage ocurred mainly in the epidermis, thus leading to a release of intracellular content. Therefore, both PEF treatments could be proposed as a pre-processing treatment of raw material to produce carrot-based products with high antioxidant potential.

Acknowledgments

This research has been financed by the Ministerio de Economía y Competitividad (Spain) reference AGL2013-44851-R. G.L.G thanks the Agència de Gestió d´Ajuts Universitaris i de Recerca (AGAUR) for the predoctoral grant.

References

Abràmoff, M. D., Hospitals, I., Magalhães, P. J., & Abràmoff, M. (2007). Image Processing with ImageJ. *Biophotonics International*, *11*(7), 36–42. https://doi.org/10.1117/1.3589100

Aguiló-Aguayo, I., Downey, G., Keenan, D. F., Lyng, J. G., Brunton, N., & Rai, D. K. (2014). Observations on the water distribution and extractable sugar content in carrot slices after pulsed electric field treatment. *Food Research International*, *64*, 18–24. https://doi.org/10.1016/j.foodres.2014.06.011

Aguiló-Aguayo, I., Gangopadhyay, N., Lyng, J. G., Brunton, N., & Rai, D. K. (2017). Impact of pulsed light on colour, carotenoid, polyacetylene and sugar content of carrot slices. *Innovative Food Science and Emerging Technologies*, *42*(May), 49–55. https://doi.org/10.1016/j.ifset.2017.05.006

Alegria, C., Pinheiro, J., Duthoit, M., Gonçalves, E. M., Moldão-Martins, M., & Abreu, M.

(2012). Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. *LWT - Food Science and Technology*, *48*(2), 197–203. https://doi.org/10.1016/j.lwt.2012.03.013

Arias, R., Lee, T. C., Logendra, L., & Janes, H. (2000). Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *Journal of Agricultural and Food Chemistry*, *48*(5), 1697–1702. https://doi.org/10.1021/jf990974e

Arscott, S. A., & Tanumihardjo, S. A. (2010). Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. *Comprehensive Reviews in Food Science and Food Safety*, *9*(2), 223–239. https://doi.org/10.1111/j.1541-4337.2009.00103.x

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., … Vorobiev, E. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International*, *77*, 773–798. https://doi.org/10.1016/j.foodres.2015.09.015

Bazhal, M., Lebovka, N., & Vorobiev, E. (2003). Optimisation of Pulsed Electric Field Strength for Electroplasmolysis of Vegetable Tissues. *Biosystems Engineering*, *86*(3), 339–345. https://doi.org/10.1016/S1537-5110(03)00139-9

Becerra-Moreno, A., Redondo-Gil, M., Benavides, J., Nair, V., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. a. (2015). Combined effect of water loss and wounding stress on gene activation of metabolic pathways associated with phenolic biosynthesis in carrot. *Frontiers in Plant Science*, *6*(October), 837. https://doi.org/10.3389/fpls.2015.00837

Berridge, M. V., Herst, P. M., & Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review*, *11*(SUPPL.), 127–152. https://doi.org/10.1016/S1387-2656(05)11004-7

Cuéllar-Villarreal, M. del R., Ortega-Hernández, E., Becerra-Moreno, A., Welti-Chanes, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2016). Effects of ultrasound treatment and storage time on the extractability and biosynthesis of nutraceuticals in carrot (Daucus carota). *Postharvest Biology and Technology*, *119*, 18–26. https://doi.org/10.1016/j.postharvbio.2016.04.013

Faridnia, F., Burritt, D. J., Bremer, P. J., & Oey, I. (2015). Innovative approach to determine the effect of pulsed electric fields on the microstructure of whole potato tubers: Use of cell viability, microscopic images and ionic leakage measurements. *Food Research International*, *77*, 556–564. https://doi.org/10.1016/j.foodres.2015.08.028

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Díaz-López, V., Artés, F., & Artés-Hernández, F. (2017). Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots. *Postharvest Biology and Technology*, *127*, 99–104. https://doi.org/10.1016/j.postharvbio.2016.12.010

Galindo, F. G., Dejmek, P., Lundgren, K., Rasmusson, A. G., Vicente, A., & Moritz, T. (2009). Metabolomic evaluation of pulsed electric field-induced stress on potato tissue. *Planta*, *230*(3), 469–479. https://doi.org/10.1007/s00425-009-0950-2

González-Casado, S., Martín-Belloso, O., Elez-Martínez, P., & Soliva-Fortuny, R. (2018). Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments: Effects on respiratory activity and quality attributes. *Postharvest Biology and Technology*, *137*(November 2017), 113–118. https://doi.org/10.1016/j.postharvbio.2017.11.017

Heredia, J. B., & Cisneros-Zevallos, L. (2009). The effects of exogenous ethylene and methyl jasmonate on the accumulation of phenolic antioxidants in selected whole and wounded fresh produce. *Food Chemistry*, *115*(4), 1500–1508. https://doi.org/10.1016/j.foodchem.2009.01.078

Jacobo-Velázquez, D. A., Cuéllar-Villarreal, M. del R., Welti-Chanes, J., Cisneros-Zevallos, L., Ramos-Parra, P. A., & Hernández-Brenes, C. (2017). Nonthermal processing technologies

as elicitors to induce the biosynthesis and accumulation of nutraceuticals in plant foods. *Trends in Food Science and Technology*, *60*, 80–87. https://doi.org/10.1016/j.tifs.2016.10.021

Jacobo-Velázquez, D. a, González-Agüero, M., & Cisneros-Zevallos, L. (2015). Cross-talk between signaling pathways: the link between plant secondary metabolite production and wounding stress response. *Scientific Reports*, *5*, 8608. https://doi.org/10.1038/srep08608

Janositz, A., Noack, A. K., & Knorr, D. (2011). Pulsed electric fields and their impact on the diffusion characteristics of potato slices. *LWT - Food Science and Technology*, *44*(9), 1939–1945. https://doi.org/10.1016/j.lwt.2011.04.006

Jin, T. Z., Yu, Y., & Gurtler, J. B. (2017). Effects of pulsed electric field processing on microbial survival, quality change and nutritional characteristics of blueberries. *LWT - Food Science and Technology*, *77*, 517–524. https://doi.org/10.1016/j.lwt.2016.12.009

Knorr, D., & Angersbach, A. (1998). Impact of high-intensity electric field pulses on plant membrane permeabilization. *Trends in Food Science and Technology*, *9*(5), 185–191. https://doi.org/10.1016/S0924-2244(98)00040-5

Lebovka, N. I., Praporscic, I., & Vorobiev, E. (2004). Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples. *Innovative Food Science and Emerging Technologies*, *5*(1), 9–16. https://doi.org/10.1016/j.ifset.2003.12.001

Leong, S. Y., & Oey, I. (2014). Effect of pulsed electric field treatment on enzyme kinetics and thermostability of endogenous ascorbic acid oxidase in carrots (Daucus carota cv. Nantes). *Food Chemistry*, *146*, 538–547. https://doi.org/10.1016/j.foodchem.2013.09.096

Martín-Belloso, O., & Elez-Martínez, P. (2005). *Food Safety Aspects of Pulsed Electric Fields*. *Emerging Technologies for Food Processing: An Overview*. Elsevier Ltd. https://doi.org/10.1016/B978-0-12-676757-5.50010-4

549 Nowacka, M., & Wedzik, M. (2016). Effect of ultrasound treatment on microstructure, colour
550 and carotenoid content in fresh and dried carrot tissue. *Applied Acoustics*, *103*, 163–171.
551 https://doi.org/10.1016/j.apacoust.2015.06.011

552 Ohshima, T., Tamura, T., & Sato, M. (2007). Influence of pulsed electric field on various
553 enzyme activities. *Journal of Electrostatics*, *65*(3), 156–161.
554 https://doi.org/10.1016/j.elstat.2006.07.005

555 Ribas-Agustí, A., Cáceres, R., Gratacós-Cubarsí, M., Sárraga, C., & Castellari, M. (2012). A
556 Validated HPLC-DAD Method for Routine Determination of Ten Phenolic Compounds in
557 Tomato Fruits. *Food Analytical Methods*, *5*(5), 1137–1144.
558 https://doi.org/10.1007/s12161-011-9355-5

559 Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future
560 needs. *LWT - Food Science and Technology*, *43*(3), 381–392.
561 https://doi.org/10.1016/j.lwt.2009.05.018

562 Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of Pulsed
563 Electric Fields on Bioactive Compounds in Foods: A Review. *Trends in Food Science &*
564 *Technology*, *20*(11–12), 544–556. https://doi.org/10.1016/j.tifs.2009.07.003

565 Soliva-Fortuny, R., Vendrell-Pacheco, M., Martín-Belloso, O., & Elez-Martínez, P. (2017).
566 Effect of pulsed electric fields on the antioxidant potential of apples stored at different
567 temperatures. *Postharvest Biology and Technology*, (March).
568 https://doi.org/10.1016/j.postharvbio.2017.03.015

569 Tiwari, B. K., Patras, A., Brunton, N., Cullen, P. J., & O'Donnell, C. P. (2010). Effect of
570 ultrasound processing on anthocyanins and color of red grape juice. *Ultrasonics*
571 *Sonochemistry*, *17*(3), 598–604. https://doi.org/10.1016/j.ultsonch.2009.10.009

572 Vallverdú-Queralt, A., Oms-Oliu, G., Odriozola-Serrano, I., Lamuela-Raventós, R. M., Martín-
573 Belloso, O., & Elez-Martínez, P. (2013). Metabolite profiling of phenolic and carotenoid

574 contents in tomatoes after moderate-intensity pulsed electric field treatments. *Food*
575 *Chemistry*, *136*(1), 199–205. https://doi.org/10.1016/j.foodchem.2012.07.108

576 Wiktor, A., Gondek, E., Jakubczyk, E., Dadan, M., Nowacka, M., Rybak, K., & Witrowa-
577 Rajchert, D. (2018). Acoustic and mechanical properties of carrot tissue treated by pulsed
578 electric field, ultrasound and combination of both. *Journal of Food Engineering*,
579 *238*(June), 12–21. https://doi.org/10.1016/j.jfoodeng.2018.06.001

580 Wiktor, A., Sledz, M., Nowacka, M., Rybak, K., Chudoba, T., Lojkowski, W., & Witrowa-
581 Rajchert, D. (2015). The impact of pulsed electric field treatment on selected bioactive
582 compound content and color of plant tissue. *Innovative Food Science and Emerging*
583 *Technologies*, *30*, 69–78. https://doi.org/10.1016/j.ifset.2015.04.004

584 Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to production
585 of plant secondary metabolites. *Biotechnology Advances*, *23*(4), 283–333.
586 https://doi.org/10.1016/j.biotechadv.2005.01.003

587